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Do natural rubber latex condoms pose a risk to aquatic systems?

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Abstract

The presence and potential adverse effects of plastic-polymers in the environment are receiving increasing attention in the popular and scientific press. However, quantifying emissions, exposure and effects of these materials remains a challenge. This paper describes the application of a questionnaire survey to quantify emissions of condom material from the domestic household to the sewage waste stream. Condoms are an important mainstay for birth control and the reduction of sexually transmitted infections. Survey participants were estimated to flush condoms down the toilet 2.96 % of the time, and emissions were calculated as 0.99 mg of condom material per person per day. Using information on screening efficiencies at sewage treatment plants, the questionnaire data was combined with a GIS-based water quality model (LF2000-WQX) to predicted environmental concentrations (PEC) in a UK river basin catchment. Annual average PECs of condom material were 0.08-0.2 µg/L, under the model scenario used. To put these PECs into context, rubber latex condom material was degraded in outdoor microcosms. This resulted in the formation of a complex mixture of substances including chemical degradation products and particles in the nano range. The direct effects of the degradation mixture were investigated using two freshwater organisms with different life cycle traits, the water column crustacean *Daphnia magna* and the sediment-dwelling larval of *Chironomus riparius*. Ecotoxicity tests investigated both acute and chronic endpoints and were shown to exhibit no toxic effects. This precluded the derivation of a genuine no-effect concentration. Hence, the results suggest that limited risk to invertebrates is associated with latex condom degradation products to the organisms tested. Future studies should extend this risk framework to assess risks of condoms to other taxonomic groups as well as the risks of other polymer materials.

Keywords: plastics, degradation products, ecotoxicity, nanoparticles, catchment modelling, environmental expose assessment

1 Introduction

Polymer-based materials (PBMs) are produced and used in a variety of commercial products. Their popular use has inevitably resulted in an increase in their release to the environment. As an environmental pollutant, bulk PBMs are identified as presenting a hazard to marine mammals and birds as they can become entangled and/or mistake them as a food source [1-4]. Once in the environment, weathering processes act to decompose PBMs. Therefore, receiving environments are potentially exposed to a mixture of the 'bulk' parent material, fragmented particles of varying sizes, leached additives and subsequent transformation products [5, 6]. Microscopic polymer particles have been reported as floating on the ocean surface, mixed into the water column, and embedded in bottom sediments and beach sands, and their uptake into a range of marine biota has previously been reported [7-10]. The formation of nano-sized polymer particles has also previously been demonstrated during the degradation of natural rubber latex (NRL) in outdoor freshwater microcosms [5]. Nanoparticles (NPs) are of particular concern as they can enter cells by endocytosis, a route available to viruses, which can then be directed to the brain and polymer NPs could potentially follow the same pathway [11]. The diversity of compounds added during the manufacturing of PBMs is also extensive. Ecotoxicological studies performed so far on these additives have focused on the endocrine disrupting potential, for example, of phthalates [12]. Degradation processes have the potential to form a complex mixture of other transformation products, some of which will be present at very low concentrations [6].

Presently, there is a growing body of research documenting the environmental occurrence of PBMs, but exact release volumes are difficult to quantify. The domestic household is a significant contributor of PBMs to the various waste streams with items disposed of through rubbish collection, and polymer-based personal hygiene products, such as condoms being disposed of to the sewage waste stream. Condoms are an important mainstay for birth control, the reduction of sexually transmitted infections, and HIV reduction [13]. Their disposal down the toilet is often seen as the most hygienic method of disposal [14, 15]. It is estimated that 1.5-2 billion sanitary protection products are disposed of via the sewage system every year in the UK, including ~ 60 million condoms [16]. Screens at wastewater treatment plants (WWTPs) have varied efficiencies at retaining solids, and the efficiency of screens is affected by the nature of the solids[17]. Larger items are generally removed by coarse screens that typically have a mesh size of 5-6 mm, with estimated screen capture ratios (SCRs) between 50-80 % depending on the screen type used whereas fine screens typically have mesh sizes of 1-3 mm with estimated removal for flushed items of > 80 % based on SCR [18]. Items disposed of to the sewage waste stream have the potential to decompose to some degree in the sewerage network. Microscopic particles and fibres can potentially pass through WWTPs and enter the environment in effluent waters [19]. Bulk items may also be released directly to surface waters during overflow events when sewage often by-passes screening processes.

An understanding of the release pathways of PBMs and the ecological effects of their degradation products is essential to understanding the risks of PBMs to environmental systems. The aim of this study was to assess the environmental risk of the degradation products formed during the breakdown of a case study PBM. The study initially quantified the emissions of condom material to surface waters, and then established the extent to which NRL condom degradates affect the viability of aquatic organisms. Exposure estimations were then compared with the ecotoxicity data to establish the risks of condoms to the environment.

2 Methods

2.1 Chemicals and materials

The NRL condom samples (0.08 mm thickness) were provided by a leading UK manufacturer. The material comprised a complex combination of constituent polymer (cross-linked *cis*-1,4-polyisoprene) and 18 other compounds including a zinc-based accelerator, an antimicrobial agent, an antioxidant, stabilizing agents, surfactants, and various other pigments and solvents.

2.2 Condom surface water exposure calculation

2.2.1 Condon use and flush rate survey

A consumer survey was performed to quantify the disposal of condoms to the sewage system. A broad survey was performed for the whole of the UK with a more intensive survey performed for the catchment used in the subsequent modelling investigations. Usage was estimated by asking respondents how often they bought condoms. For the purposes of this survey it was assumed that all condoms purchased are used. Condom sales figures often refer to sales to wholesalers and distributors, and will include condoms that are in stock at various levels in the supply chain [20], potentially leading to an overestimation of usage. Qualitative data on condom disposal to the sewage system was obtained by asking respondents how often they disposed of condoms down the toilet. Information about age, sex, education, and living situation were collected to assess population demographics. Respondents were also asked to provide the first part of their postcode to allow catchment scale analysis. Only closed-ended questions were used, with specified possible answers supplied. These types of questions are easier to answer, and easier to analyse and interpret than open-ended questions [21]. The survey was performed in July/August 2012 and lasted for 3 weeks. Invitations to participate were

distributed across social networking sites, universities, work places, town councils and Rotary Clubs. To minimise respondents feeling pressured to give the answers they think are correct, or those they think the questioner wanted to hear, participants were asked to complete the questionnaire survey online using Survey Monkey (http://www.surveymonkey.com/). This also enabled the information to be collected anonymously and without respondents having to feel embarrassed about the survey content.

2.2.2 Estimation of daily emissions to the sewage system

Daily per capita emissions (mg/person/day) of parent condom material disposed to the sewage system were calculated using the survey purchase and flush rate data, as well as data collected on product weight and pack size. The mass of condom material (mg) purchased per person per day was calculated using Equation 1.

$$M = \frac{pd \text{ x } ps \text{ x } w}{SurvPop \text{ x } t} \qquad \text{Equation 1}$$

Where: *M* is purchase behaviour (mg/capita/day), *pd* is the purchase data (i.e. number of packs bought per year by the survey population), *ps* is pack size specified in the survey to be 12, *w* is condom weight (1312 mg ± 5.31 mg), *SurvPop* is the survey population, and *t* is days per year.

Condom flush rate (%) was calculated by converting qualitative emission information on flush rates (i.e. never, occasionally, sometimes, most of the time, always) to quantitative data (0 %, 25 %, 50 %, 75 %, 100 % respectively) based on a 5-point Likert scale approach [21] (Equation 2).

$$FR = \frac{N_{25}\text{x } 0.25 + N_{50} \text{ x } 0.5 + N_{75} \text{ x } 0.75 + N_{100} \text{ x } 1}{N_0 + N_{25} + N_{50} + N_{75} + N_{100}} \text{ X } 100 \qquad \text{Equation 2}$$

Where: FR is the condom flush rate (%), N_X is the number of people that flush at rate x %. Please refer to the supplementary material for the raw data.

Daily per capita emissions (mg/person/day) of condom material disposed to the sewage system was then calculated using Equation 3.

$$E = \frac{M}{100} \text{ x } FR \qquad \text{Equation 3}$$

Where: E (mg/capita/day) is the emissions to the sewage system.

2.2.3 Surface water exposure modelling

Predicted environmental concentrations (PECs) in surface water for parent condom material were derived using the LF2000-WQX model, continuous flow scenario (LowFlow2000-Water Quality eXtension; Centre for Ecology & Hydrology, Wallingford, UK). The modelling was undertaken on a catchment scale basis and focused on the rivers Ouse, Derwent and Aire, which flows through the north east of England (Dales and Riding region) and discharge to the North Sea via the Humber Estuary. The model is a geographical information based system that assesses the spatial exposure of contaminants in surface waters by combining hydrological models with a catchment scale water-quality model. Spatially explicit statistical distributions of down the drain chemicals are generated using a Monte Carlo mixing-model approach to combine statistical estimates of chemical loads at specific emission points (e.g. WWTPs). The model starts at the low order streams at the head of the river network and works towards the outlet of the river basin, and accounts for the accumulation of point loads and the accumulation of water in which these loads are diluted [22]. For the purposes of this model, the removal for flushed condoms at WWTPs was based on reported SCRs (50-80 %) [18]. Flushed items were classed as a conservative waste type with no in-stream removal, assuming all items are transported downstream with no degradation. The

model has previously been used to assess concentrations of steroid estrogens [22], cytotoxic drugs [23], glucocorticoids [24], and triclosan [25] in UK surface waters.

2.3 Toxicity of NRL degradates

2.3.1 Generation of NRL degradates

To produce test material for use in the ecotoxicity tests, NRL condom material was exposed to natural conditions in outdoor microcosms. Microcosms were established using stainless steel containers, and were covered with a non-ultra-violet filtering perspex sheet (B&Q, UK) to prevent flooding by rainfall. Evaporation was dealt with by regularly adding demineralised water to the desired level. Condom material was exposed in the microcosms for 1, 3, 7, 14, 28, 56, 112 and 200 days starting from June 2011. Each time point consisted of two treatment groups: one with the presence of NRL material, and the second with the absence of NRL material. This second treatment group therefore acted as a test system control. The concentration of NRL condom material was 0.75 g / L demineralised water. This ratio was used to mirror the NRL-to-water ratio used in our previous outdoor microcosm experiments that were designed to characterise the NRL degradation process [5, 6]. Upon collection, the bulk NRL material was removed by using a 2 mm sieve, followed by filtration using a 1.6 µm pore diameter glass fibre paper (Whatman, UK). The filtrate was then stored in Nalgene wide mouth PP bottles at -22 °C for use in the ecotoxicity studies, with a subsample taken for analysis.

2.3.2 Analysis of degradate solutions

Nanoparticle tracking analysis (NTA) was used to determine the number and size distribution of NRL particles (30 nm to 2000 nm) formed during the degradation process. Analysis was performed using NanoSight LM 10 (NanoSight Ltd, UK). Previous studies have shown this

technique to be suitable for characterisation of samples with heterogenous distributions of NPs as it does not give bias towards larger particles [26, 27]. To characterise each sample generated in a representative manner, six video images of each sample were taken at room temperature. The focus of the camera was judged by eye and was adjusted so the majority of particles on the screen were in focus at the start of video capturing. Video image length was set at 30 s. Processing of video images was performed using NTA 2.2 software. The detection threshold was set to automatic; this determines the minimum grey scale value of any particle in the image necessary for it to qualify as a particle to be tracked. A blur (smoothing setting) of 5 x 5 was applied following the recommendation in the operating manual that if automatic threshold detection is used, the blur setting should be increased by one level. The minimum expected particle size was set at 30 nm. The minimum track length, which defines the minimum number of steps a particle must take before its size is calculated and included in the analysis was set to automatic, allowing the software to calculate this based on the particles in the video.

The mass of particles present was estimated using the NTA distribution data. This was done by calculating the volume of particles present in the sample (Equation 4) and then multiplying by the density (ρ) of the solid which was taken to be 920 mg/cm^3 for polyisoprene (Equation 5). It should be noted that for this calculation it was assumed all particles were solid and spherical in shape.

Volume (cm^3/ml) = $\frac{\pi}{6}d^3$ (cm) x concentration (no. particles / ml) Equation 4

Mass (mg/ml) = Volume x ρ Equation 5

2.3.3 Test organisms and their cultivation

The test organisms were chosen to represent freshwater aquatic species with different life cycle traits. The freshwater crustacean *Daphnia magna* lives in the water column and as a filter-feeding organism can catch suspended particles and may inadvertently ingest foreign materials from surrounding water [28]. The second test organism was the sediment larva of the aquatic midge *Chironomus riparius*. Both organisms hold an important position in aquatic food webs, are distributed globally, and are used extensively to assess both acute and chronic toxicities of sediment and water pollutants [29, 30].

D. magna Straus were cultured in M4 medium, at a constant temperature (20 ± 1 ^{o}C) with a 16-8 h light-dark photoperiod, and a light intensity of 15-19 μE/m^{2}s. The cultures were renewed using offspring of four-week old daphnids and consisted of 2 L glass beakers containing 1.5 L of culture media, with 20-25 daphnids. Neonates were removed three times a week and the culture medium was renewed once a week. Daphnids were fed with the green algae *Desmodermus subspicatus* three times a week with 0.14 mg TOC per daphnid. *D subspicatus* used for feeding was cultivated using Kuhl-medium for 2 weeks in 1 L media bottles, under continuous aeration, constant temperature, and constant light with an intensity of 45 μE/m^{2}s. *C. riparius* were cultured in an insect breeding and rearing cage aquarium (BugDrom) using artificial freshwater medium ($CaCl_2$ 294 mg/L; $MgSO_4$ 123.25 mg/L; $NaHCO_3$ 64.75 mg/L and KCl 5.75 mg/L), at a constant temperature (20 ± 1 ^{o}C), with a 16-8 h light-dark photoperiod, and constant aeration. Cultures were fed with fish flake food (Tetramin, Tetrawerke, Melle, Germany) at a rate of 0.5 mg per larvae per day.

2.3.4 Toxicity of NRL degradates

The aquatic ecotoxicity of NRL degradates was tested, using both acute and chronic endpoints, by comparing the effect of those solutions with the presence of NRL degradates to those without, at various dilutions.

Acute toxicity tests: *D. magna* acute toxicity tests were performed under the same conditions used for the cultures. All sampling time points were tested and involved six test dilutions (0, 10, 25, 50, 100, 200, 500 ml / L M4 media). Each test dilution was made up of five replicates, and each replicate consisted of five *D. magna* neonates (< 24 h) in 20 ml glass vessels with 10 ml test solution. The test vessels were covered with a glass lid and the number of immobile organisms was counted after 48 h.

Chronic toxicity tests: *D. magna* reproduction and growth was assessed in a semi-static test design, using t = 56 and 112 day NRL degradate samples only. The tests were performed under the same conditions as the culture. Daphnid aged < 24 h at the start of the test were exposed for a period of 21 days to a set of seven test dilutions (0, 10, 50, 100, 200, 350 and 500 ml/L M4 medium). Each test dilution consisted of 5 test organisms held individually in 80 ml test solution. Test solutions were renewed once a week. To assess impacts on reproduction, neonates were removed from test vessels and counted daily. To measure impacts on growth, body length was measured at the start and end of the exposure period using an incident light scanner (Canon, CanoScan 8800F) at a resolution of 1200 dpi. To do this, individual organisms were transferred to a Petri dish and any media was removed until organisms were observed to display minimal movement. The images were then analysed with purposely designed software (T.G. Preuss, Institute for Environmental Research, Aachen, Germany). Body length was defined as from the top of the eye to the base of the spine following Agatz et al., [31].

C. riparius emergence was assessed using a static test design using the t = 200 day NRL degradate sample only. The test was performed under the same conditions to those under which the culture was kept. Egg masses were obtained from cultures and transferred into vessels containing culture medium. First instar larvae (1-4 days post hatching) were used. Hatched larvae were exposed for 28 days to a set of six test concentrations (1, 10, 50, 100,

200, 500 ml/L) made up in artificial freshwater medium. Each treatment consisted of 20 larvae held together in 500 ml beakers with sediment added to a depth of 3 cm. Sediment was prepared according to OECD 219. Test vessels were capped with 473 ml plastic food containers and aeration was provided using an aquarium pump through a Pasteur pipette via suitable tubing and placed so as not to disturb the sediment. Media was replenished daily to avoid desiccation. To assess impacts on emergence, organisms were removed from test vessels and counted daily.

2.4 Statistical analysis

A two-way ANOVA was utilised to compare immobility, reproduction, growth and emergence across concentrations and treatment groups. All pairwise multiple comparisons were conducted using Tukey test. Prior to all tests, data were tested for normality and equal variance by utilising a Shapiro-Wilk and Levene-Mediane test respectively. When comparing NRL particle concentration between the NRL degradates samples (with and without the presence of NRL), both normality and equal variance tests failed so the non-parametric Friedman test was used. All statistical analysis was performed using SigmaPlot version 12 and a significance level of 0.05.

2.5 Risk characterisation

To estimate the environmental risk of NRL degradation products a strategy was adopted that compares the PECs for the parent material, to the predicted no-effect concentrations (PNECs) of the NRL degradates. PNECs of the NRL degradates were be derived from the chronic no-observed effect concentrations (NOECs) by applying an assessment factor (AF) of 100 [32]. The overall risk is then characterised using the traditional risk ratio by calculating PEC/PNEC ratios.

3. Results

3.1 Survey results and modelled concentrations of condoms in surface waters

The survey generated 387 respondents of which 152 were identified as based in the study catchment. In the catchment population gender was split 46.7 % male and 53.3 % female, and closely reflected the UK distribution (51.2 % male, 48.8 % female). The respondents were evenly spread across age range and the dominant household category was family home (see supplementary material). The results generated indicate that individuals will flush condoms down the toilet 2.96 % of the time, so the calculated input of condom material to the sewage waste stream was 0.99 mg/person/d.

The results collected for the catchment population compared well with those collected for the overall population surveyed, which were as follows: gender for the whole population surveyed was split 45.7 % male and 54.3 % female, and individuals flushed condoms down the toilet 2.97 % of the time. The input of condom material to the sewage waste stream was therefore 1.07 mg/person/d, for the whole survey population (see supplementary material).

PECs for NRL material were predicted for rivers in the Ouse and Derwent catchment. When assuming 50 and 80 % screening efficiency by WWTPs, annual mean PECs were 0.2 µg/L (± 0.31) for the 50% removal scenario and 0.08 µg/L (± 0.12) for the 80% removal scenario with maximum PECs of 1.67 µg/L and 0.67 µg/L respectively (Fig. 1). The annual mean NRL concentrations for catchment surface waters were greatest for the rivers Aire and Calder downstream from more highly populated locations in the catchment such as Leeds and Halifax. The river Nidd was highlighted as having river stretches with the highest concentrations. The rivers Derwent, Ouse, Swale, Ure and Wharfe had lower predicted concentrations for both scenarios as the surrounding areas are predominately agricultural.

3.2 NRL degradate particle analysis

Concentrations of NRL particles in the degradate mixture increased over time when compared to the aged water samples (Fig. 2, $\chi^2 = 6.75$, $p = 0.009$). Generally, mean particle diameter remained constant, but became more variable over time. Overall, the sizes of particles formed were mainly in the 100 – 500 nm range. The mass of NRL particles present was generally seen to increase in line with particle concentration ($r^2 = 0.974$, $p < 0.001$).

3.3 NRL degradate toxicity

The generated treatment solutions were not acutely toxic to *D. magna* when immobility in the presence and absence of NRL degradates was compared for each time point (Table 1). The chronic toxicity tests also showed no differences in the growth of *D. magna* when the presence and absence of NRL degradates was compared for the time points tested ($t = 56$, $p = 0.73$, F = 0.121 and $t = 112$, $p = 0.188$, F = 1.792; Fig. 3b). Reproduction was identified to be greater in the presence of degradation products for $t = 56$ days ($p < 0.001$, F = 33.742; Fig.3a). This is potentially due to the increased concentration of organic carbon in this mixture, originating from the degraded NRL. It is assumed that the organisms may use this additional carbon as a food source, enhancing their reproductive output. For samples aged $t = 112$ days no differences in reproduction between the two treatment groups were identified ($p = 0.483$, F = 0.501; Fig.3). The *D. magna* mortality observed at the high concentrations are more than likely explained by the effect caused by the dilution of the growth media. In view of the results the remaining samples were not tested.

There was no concentration-dependent decrease in emergence of *C. riparius* after 28 days exposure to the two treatment groups ($t = 200$ days, $p = 0.495$, F = 0.561; Fig.3c). Due to the results obtained EC_{50} values for reproduction and growth (*D. magna*), and emergence (*C.*

riparius) could not be calculated. Therefore, investigation did not proceed further than these initial range finding tests and other generated samples were not tested.

3.4 Risk characterisation

To estimate the worst case scenario the calculated PEC can be based on the modelled maximum concentrations for the two scenarios investigated that assumed 50 and 80 % removal (1.67 µg/L and 0.67 µg/L, Fig. 1). Results of our previous NRL fate investigations, which were performed alongside this study, were used to convert the dilutions used into NRL degradate concentrations (mg/L; Table 2, also see supporting information table 3). The PECs for NRL condom material were clearly much lower than the highly conservative PNEC value. Hence, the risk ratio for surface water was well below 1, indicating no significant risk to aquatic invertebrates is expected from NRL condom degradation products.

4. Discussion

The sources from which PBMs enter the environment and their importance are known to differ between geographical locations depending on public behaviour and the infrastructure present [33]. However, the environmental releases of PBMs are difficult to quantify, because inadvertent littering is problematic to monitor, and in general people do not want to admit to littering or dumping of unwanted waste. This is the first study to quantify the environmental release of a case-study PBM, and then to combine this with long-term effect data for naturally weathered degradation products. Condoms are an interesting case study material as they are a personal care product with a complex chemical makeup and a high market turnover. Factors that may influence condom usage and disposal habits are the frequency of intercourse and the probability of a condom being used, which are in turn influenced by a range of socio-demographic factors, such as, age, sexual orientation and education [20, 34]. The major rivers in the catchment system have a number of sewage treatment facilities and combined sewage

overflows that will receive wastewater discharges from both domestic and industrial effluents. The occurrence of sewage related debris (SRD) and residues of such materials in river systems can vary and depend on demographics, individuals flushing habits and the types of items flushed. The surface water exposure modelling undertaken in this study showed that predicted environmental concentrations (PEC) are low. However, the occurrence of SRD is noted as a problem on British beaches were it can accounted for 4.5 % of total waste [35]

Previous fate investigations have shown that NRL degrades into a complex makeup of the substances[5, 6]. Therefore, it was not possible to test the effects of the individual components of the mixture. In this case it was decided to test the direct toxicity of the NRL degradate mixture. The results of the ecotoxicity tests precluded the derivation of a genuine PNEC. However, none of the samples generated were shown to have a toxic effect, even though zinc concentrations were potentially within the range (0.05 mg/L to 2.5 mg/L) reported as causing long-term effects on microbenthic communities [36-38]. Zinc toxicity is generally associated with the presence of free zinc. The zinc measured here is more likely associated with zinc-based accelerators and activator compounds. The presence of humic substances in the degradation mixture may also help to mitigate adverse effects. Humic substances are thought to influence zinc toxicity by decreasing the amount of free metal ions though zinc–humic acid complexes, these complexes have high molecular weight, are relatively stable with regard to metal-exchange reactions, and consequently the metals were less bioavailable [39, 40]. Overall, these results largely agree with Lither et al., [41] who conducted an extensive acute screening of leachates from new PBM consumer products to *D. magna* for 32 products, and found 48 h EC_{50} of leachates ranged from 5-80 g of material/L.

An additional concern was organism exposure to particles < 1.6 µm. Studies using engineered nanoparticles (ENPs), such as, nano-TiO_2 [42], quantum dots [43], nano-Ag [44], and carbon nano-tubes [45], have shown uptake by *D. magna*. In this case, uptake of the particulate component was not monitored, but the chronic *D. magna* and *C. riparius* tests did not show any effects up to the highest concentrations tested. The exposure of the test organisms to the complex mixture of NRL degradation products was more reflective of realistic environmental exposure scenarios, and the impacts take into account the interacting effects of both particulate and chemical degradates. Future studies may want to monitor effects towards fish species, because latex NPs (39 nm) have been observed to accumulate in fish gills when exposed to NP concentrations of 10 mg/L, and were also detected in the brain, liver and blood of *Oryzias latipes* [46].

5. Conclusion

Predicting environmental concentrations of PBMs represents a challenge for the scientific community. The present study attempted to quantify the emission of a case study polymer emitted from the domestic household through the sewage waste stream. This information was then used to predict potential environmental concentrations of condom material at a catchment scale. To put the predicted concentrations into context the effects of NRL degradates to two freshwater organisms, one whose live cycle is spent in the water column and one which exhibits a sediment larval stage, were investigated. The NRL degradate samples used, as far as possible, were degraded under environmentally relevant conditions, and produced a complex mixture of particulate, non-soluble, and dissolved substances. The results were conclusive in that no detrimental effect as a consequence of exposure to NRL degradates was observed. As the concentrations used were orders of magnitude higher than the modelled surface water concentrations, it can be assumed that NRL degradates pose

limited environmental risk to invertebrate communities. It is recommend that future tests are conducted on a wider range of taxonomic groups and by widening the range of PBMs used by identifying those that have a large production volume, high content of additives, and high potential for additives to have an adverse effect. This study provides a framework that can be used in these studies to characterise the impacts of PBMs to the natural environment.

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Fig. 1 Distribution of predicted parent NRL condom concentrations (µg/L) across the Ouse and Derwent region of England using estimations of WWTP loadings: (a) annual average concentrations after applying a 50 % screening efficiency (map identifies the major urban centres); (b) annual average concentrations after applying a 80 % screening efficiency (map identifies the major catchment rivers).

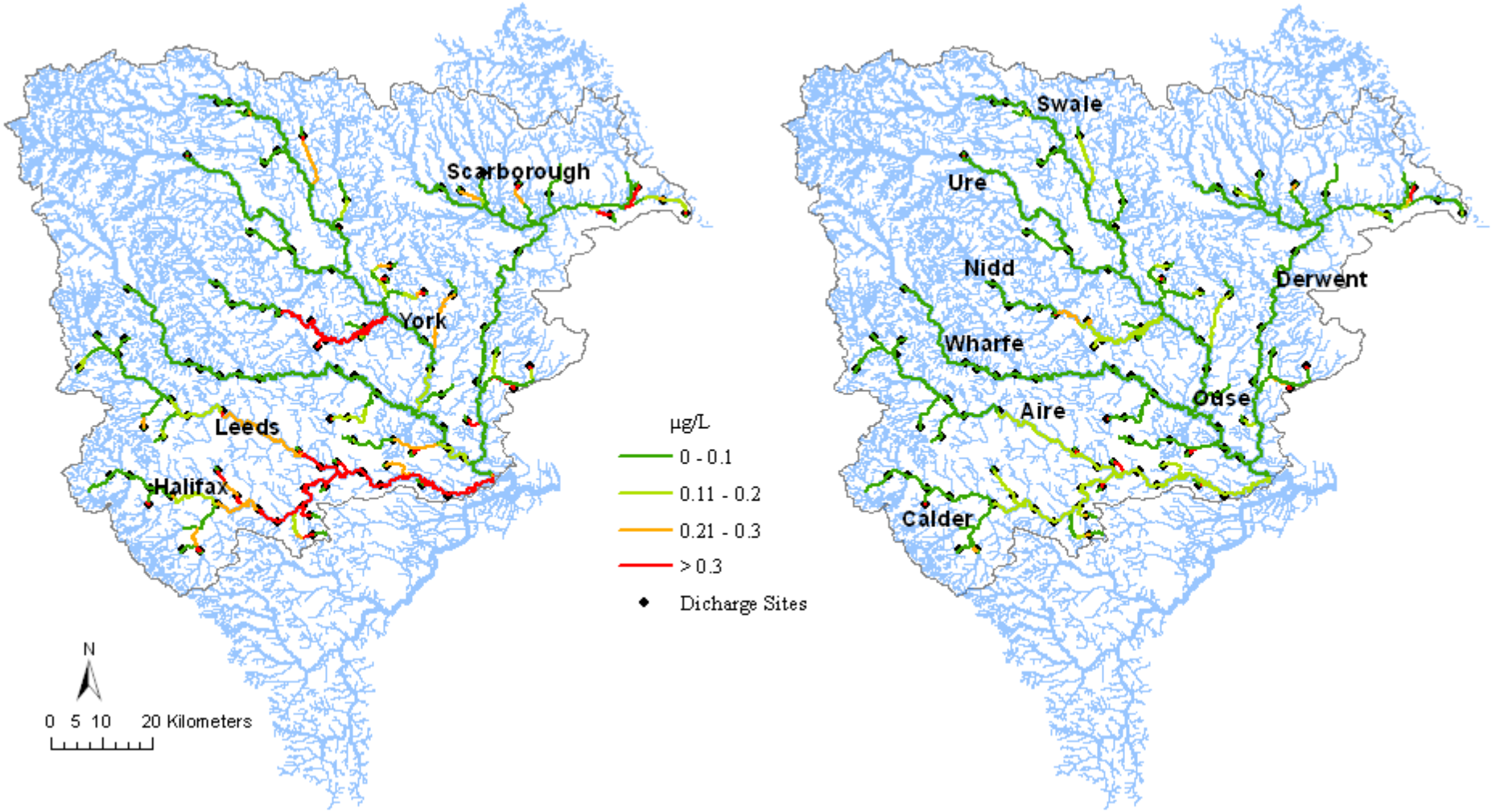

Fig. 2 Characterisation of nano sized particles formed during the degradation of natural rubber latex in outdoor microcosms. Solid bars represent particle concentration in the sample filtrate; bars with diagonal lines represent particle concentration in the controls; circles represent mean particles diameter in the samples and triangles represent particle weight. Error bars represent standard deviations for six replicates.

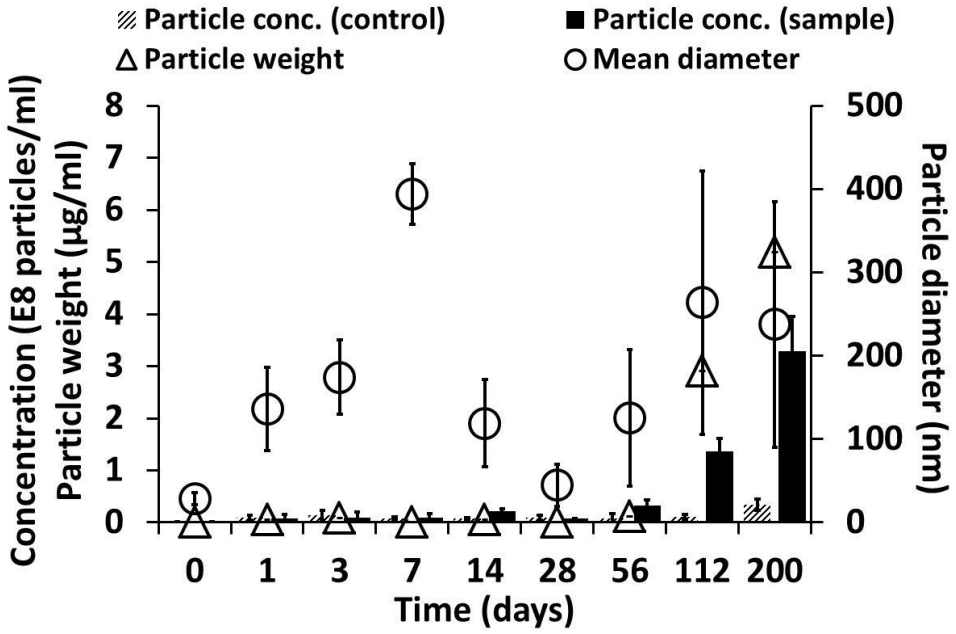

Fig. 3 Chronic effects of NRL degradates on reproduction (A) and growth (B) to *D. magna*, and on emergence (C) to *C. riparius*. Diamonds equal absence and squares equal presence of NRL degradates aged 112 days, and triangles equal absence and circles equal presence of NRL degradates aged 56 days (A and B). Solid bars equal absence and hollow bars equal presence of NRL degradates (C).

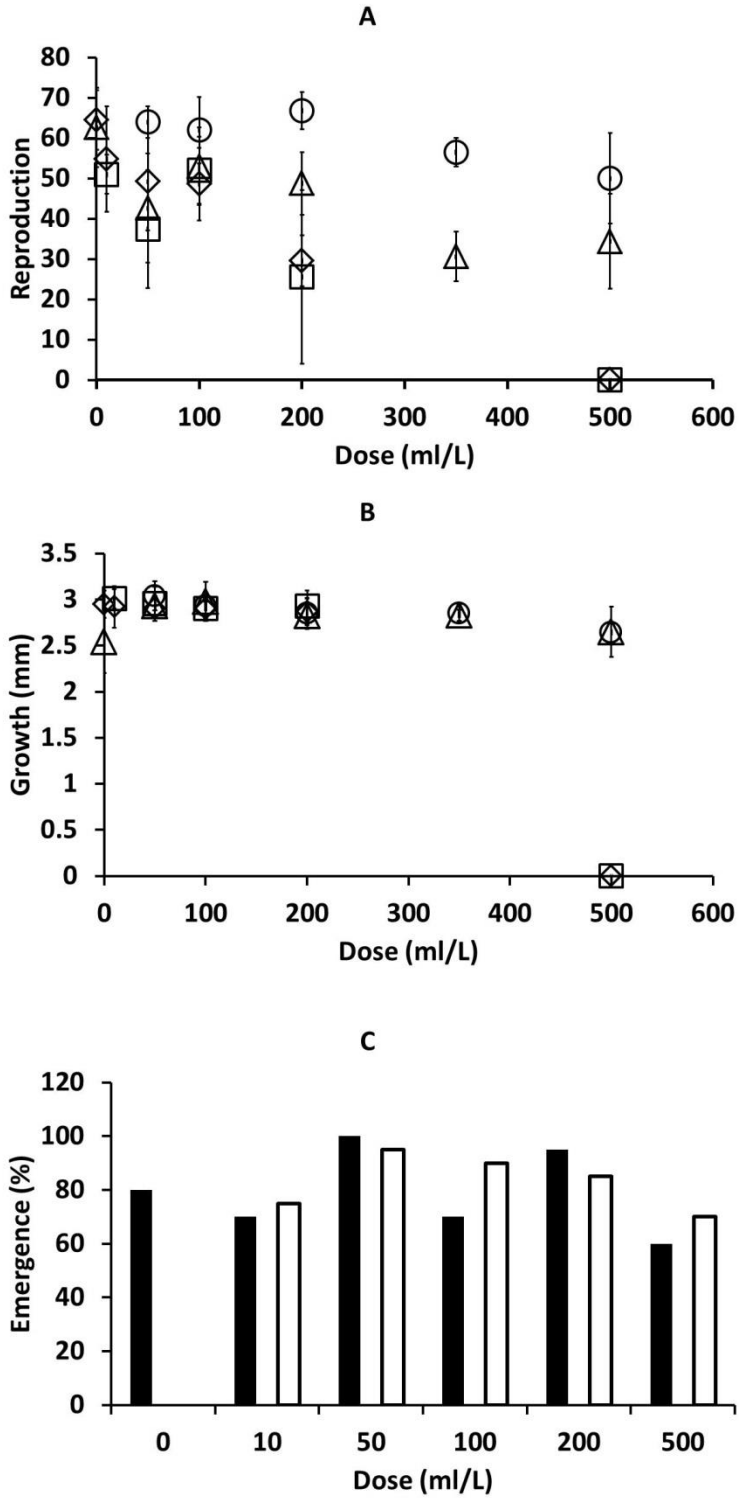

Table 1. The effect for acute and chronic toxicity of latex degradation products to *D. magna* and *C. riparius*.

Time (days)	Acute endpoint Immobility *D. magna* NOEC based on DP conc. (ml/L)	Chronic endpoint Reproduction *D. magna* NOEC based on DP conc. (ml/L)	Growth *D. magna* NOEC based on DP conc. (ml/L)	Emergence *C. riparius* NOEC based on DP conc. (ml/L)
1	> 500			
3	> 500			
7	> 500			
14	> 500			
28	> 500			
56	> 500	> 500	> 500	
112	> 500	> 500	> 500	
200	> 500			> 500

Table 2. The concentrations of NRL degradates (mg/L) present in each test dilution (ml/L) for each sampling time point. The concentrations presented are based on the concentrations of dissolved organic carbon and zinc measured in previous fate studies (see supplementary information).

	Equivalent concentration of degradation products (mg/L)							
Test dilutions (ml/L)	*t* = 200 d	*t* = 112 d	*t* = 56 d	*t* = 28 d	*t* = 14 d	*t* = 7 d	*t* = 3 d	*t* = 1 d
10	2.39	2.16	2.32	1.34	0.32	0.17	0.13	0.14
25	5.99	5.419	5.80	3.36	0.80	0.44	0.34	0.35
50	11.98	10.83	11.61	6.72	1.61	0.89	0.68	0.71
100	23.97	21.67	23.23	13.45	3.22	1.78	1.36	1.42
200	47.95	43.35	46.46	26.90	6.44	3.57	2.72	2.84
350	83.92	75.87	81.31	47.08	11.28	6.26	4.77	4.98
500	119.89	108.38	116.16	67.25	16.11	8.94	6.82	7.12

Supplementary material

Table 1. Usage and flush profile data

Flush rate (%)	Number (catchment population)	Number (overall population)
Never - 0	142	364
Occasionally - 25	7	15
Sometimes - 50	0	0
Most of the time - 75	1	1
Always - 100	2	7
Responses	152	387

Data	Unit (catchment population)	Unit (overall population)
Flush profile (%)	2.96	2.97
Number of packs purchased per year	118	322
Pack size	12	12
Condom weight (mg)	1312	1312
Daily per capita emissions (mg/person/d)	0.99	1.066

Table 2. Demographic data for the survey respondents (include housing type)

Age range	**16-20**	**21-25**	**26-30**	**31-35**	**36-40**	**41-50**	**51-60**	**61-70**	**70 +**
Catchment population (%)	4.6	19.1	11.2	12.5	6.6	11.8	12.5	15.1	6.6
Overall population (%)	5.2	23	10.6	14.7	8	15.8	11.9	7.5	3.4
Housing category	**Family home**	**Live alone**	**Multiple occupancy**	**Other**					
Catchment population (%)	57.9	11.2	27.6	3.2					
Overall population (%)	63	9.8	22.7	4.4					

Table 3. Data from previous work used to convert the dilutions used in this work into NRL degradate concentrations (mg/L).

Time (days)	DOC (mg/L)	Zinc (mg/L)	Total NRL degradates
1	13.54	0.70	14.24
3	13.06	0.58	13.64
7	17.34	0.55	17.89
14	31.58	0.65	32.23
28	133.84	0.67	134.52
56	231.39	0.95	232.34
112	215.52	1.26	216.78
200	237.83	1.95	239.78